Owen, W. J., Rogers, L. J. & Hayes, J. D. (1970). *Biochem. J.* **121**, 6 P.

Yamashita, T. & Butler, W. (1968). *Pl. Physiol., Lancaster*, **43**, 1978.

Yamashita, T. & Butler, W. (1969). *Pl. Physiol., Lancaster*, **44**, 435.

Partial Purification from Rat Liver of an Enzyme Catalysing the Sulphurylation of Dehydroepiandrosterone

By R. RYAN and J. CARROLL. (*Department of Biochemistry, Trinity College, Dublin 2, Irish Republic*)

The sulphotransferases of mammalian tissues catalyse the transfer of the sulphuryl group of adenosine 3'-phosphate 5'-sulphatophosphate to a variety of acceptors, which include phenol, dehydroepiandrosterone, oestrone, L-tyrosine methyl ester and serotonin. None of these sulphotransferases has been obtained in a pure state, but Banerjee & Roy (1966) described the partial separation from guinea-pig liver of a number of sulphotransferases. They achieved a 20- and 70-fold increase in the specific sulphurylating activities towards dehydroepiandrosterone and oestrone respectively, but both of these fractions were active towards phenol. Mattock & Jones (1970) purified from female rat liver an enzyme that catalysed the sulphurylation of L-tyrosine methyl ester, but this purified preparation also catalysed the synthesis of *p*-nitrophenyl sulphate. McEvoy & Carroll (1970) described the isolation from male rat liver of an enzyme that catalysed the sulphurylation of phenols but had no activity towards steroids, L-tyrosine methyl ester or serotonin. Adams & Poulos (1967) isolated from bovine adrenals an oestrogen sulphotransferase that lacked activity towards 3β-hydroxy steroids and *p*-nitrophenol. The present communication describes the partial purification from rat liver of a steroid sulphotransferase with high activity towards dehydroepiandrosterone and weak or no activity towards other acceptors.

Livers from female Wistar rats were homogenized in 0.15M-potassium chloride with a Potter–Elvehjem homogenizer, and centrifuged at 100 000*g* for 1h. The resulting supernatant was made 1mM with respect to 2-mercaptoethanol and diluted to a protein concentration of 10mg/ml. The sulphotransferase was precipitated by the addition of cold ethanol to a concentration of 25% (v/v). The precipitated protein was dissolved in 5mM-tris–HCl buffer, pH8.0, and stirred at 2°C for 1h. After centrifugation at 25 000*g* for 15min, the supernatant was collected and adjusted to 55% saturation with respect to ammonium sulphate. The precipitated protein was dissolved in 5mM-tris–HCl buffer, pH8.0, and dialysed against large volumes of the same buffer. During the dialysis inactive protein was precipitated and was discarded. Further purification was achieved by chromatography on columns of DEAE-cellulose and phosphocellulose P-11, and gel filtration on Sephadex G-200. The specific activity towards dehydroepiandrosterone of this partially purified sulphotransferase was 60-fold higher than that of the crude supernatant, and the enzyme had negligible activity towards *p*-nitrophenol, L-tyrosine methyl ester and serotonin.

Adams, J. B. & Poulos, A. (1967). *Biochim. biophys. Acta*, **146**, 493.

Banerjee, R. K. & Roy, A. B. (1966). *Molec. Pharmac.* **2**, 56.

McEvoy, F. A. & Carroll, J. (1970). *Biochem. J.* **119**, 26 P.

Mattock, P. & Jones, J. G. (1970). *Biochem. J.* **116**, 797.

A Heparan Sulphate Sulphotransferase of Ox Lung

By T. FOLEY and J. R. BAKER. (*Department of Biochemistry, Trinity College, Dublin 2, Irish Republic*)

Suzuki & Strominger (1960) first showed that a preparation from hen oviduct could catalyse the sulphation of heparan sulphate by adenosine 3'-phosphate 5'-sulphatophosphate. Suzuki, Trenn & Strominger (1961) reported the partial purification and stability of the enzyme. Using similar methods, we have extended their studies. In seeking a more convenient source of the enzyme, a study was made of its distribution in rat tissues. Marked activity was detected in lung and brain homogenates only. For purposes of purification, ox lung was the sole source of the enzyme.

Experiments were designed to determine whether the enzyme was responsible for synthesizing *N*-sulphate or *O*-sulphate groups in heparan sulphate. [Both types of sulphate ester are present in this glycosaminoglycan (Brown, 1957).] Chemically de-*N*-sulphated heparan sulphate is a better substrate for the enzyme than is heparan sulphate, suggesting that enzymic *N*-sulphation is more likely. [^{35}S]Sulphate was enzymically transferred from adenosine 3'-phosphate 5'[^{35}S]-sulphatophosphate to de-*N*-sulphated heparan sulphate. The product was purified, and unsubstituted amino groups were acetylated, before incubation with a crude 'heparinase' from *Flavobacterium heparinum*. A paper-chromatographic separation of the resulting incubation mixture in an isobutyric acid–ammonia solvent system (Suzuki & Strominger, 1960) revealed only two radioactive areas. The major ^{35}S-labelled component had the same R_F

value as authentic 2-deoxy-2-sulphoamino-D-glucose. The electrophoretic mobility of this ^{35}S-labelled product also coincided with that of 2-deoxy-2-sulphoamino-D-glucose.

The enzymically prepared heparan [^{35}S]sulphate was degraded with nitrous acid (Lagunoff & Warren, 1962) and the products were fractionated on a Sephadex G-15 column. Approx. 75% of the radioactivity was eluted with inorganic sulphate. These results suggest that predominantly N-sulphate groups are synthesized in the presence of the ox lung enzyme.

Brown, D. H. (1957). *Proc. natn. Acad. Sci. U.S.A.* **43**, 783.

Lagunoff, D. & Warren, G. (1962). *Archs Biochem. Biophys.* **99**, 396.

Suzuki, S. & Strominger, J. (1960). *J. biol. Chem.* **235**, 257.

Suzuki, S., Trenn, R. H. & Strominger, J. (1961). *Biochim. biophys. Acta,* **50**, 169.

Investigation of the Metabolic Oxidation of Acenaphthen-1-ol

By C. J. SIMMONS, R. P. HOPKINS and P. CALLAGHAN. (*Department of Biochemistry, St Thomas's Hospital Medical School, London S.E.1, U.K.*

After the administration of acenaphthene, acenaphthylene and *cis*- and *trans*-acenaphthene-1,2-diol to rats, 1,8-naphthalic acid was isolated, as its anhydride, from the acidified urine of the dosed animals (Chang & Young, 1943; Hopkins, Brooks & Young, 1962; Hopkins & Young, 1966). Following the suggestion of Hopkins (1968) that oxidation of the acenaphthene-1,2-diols to 1,8-naphthalic acid might involve an initial dehydrogenation step, acenaphthenequinone was isolated from a microsomal incubation of the acenaphthene-1,2-diols in the presence of NAD$^+$ (Drummond & Hopkins, 1969). Drummond, Callaghan & Hopkins (1971) have demonstrated that there are at least two systems in rat liver that dehydrogenate acenaphthene-1,2-diol, one in the microsomal fraction and another in the soluble fraction. These findings and the work of Sims (1966), on the structurally related 3-methylcholanthrene, led us to investigate the metabolism of acenaphthen-1-ol in order to determine whether it proceeds by a pathway involving an initial dehydrogenation reaction.

Rats were injected subcutaneously with acenaphthen-1-ol and the urine was acidified to pH 2 and extracted with ether. Examination of the extract by t.l.c. indicated the presence of acenaphthen-1-one and 1,8-naphthalic anhydride, which were isolated and characterized. 1,8-Naphthalic anhydride was also isolated and characterized from urine obtained from rats that had been dosed with

acenaphthen-1-one. Hydrolysis of the extracted urines with 6 M-hydrochloric acid or β-glucuronidase gave rise to further quantities of acenaphthen-1-one and 1,8-naphthalic anhydride.

The oxidation of acenaphthen-1-ol was also investigated by incubation at 37°C with a rat liver microsomal preparation and NAD$^+$ at pH 7.4. Ether extracts of the incubation system, after acidification to pH 2, were examined by t.l.c. and found to contain acenaphthen-1-one, which was isolated and characterized. This reaction was followed in a similar incubation system by measuring the increase in E_{340} due to accumulation of acenaphthen-1-one. The microsomal dehydrogenase system was found to use either NAD$^+$ or NADP$^+$ as cofactor.

It was also found that a rat liver microsomal fraction incubated at 37°C with an NADPH-generating system at pH 7.4 converted acenaphthen-1-one into acenaphthenequinone, which was identified by t.l.c. and g.l.c. of ether extracts of the incubation medium after acidification to pH 2. Acenaphthenequinone was isolated and characterized.

These observations will be discussed and a metabolic sequence suggested for the conversion of acenaphthen-1-ol into 1,8-naphthalic acid.

Chang, L. H. & Young, L. (1943). *J. biol. Chem.* **151**, 87.

Drummond, E. C., Callaghan, P. & Hopkins, R. P. (1971). *Biochem. J.* **121**, 4P.

Drummond, E. C. & Hopkins, R. P. (1969). *Biochem. J.* **113**, 13P.

Hopkins, R. P. (1968). *Biochem. J.* **108**, 577.

Hopkins, R. P., Brooks, C. J. W. & Young, L. (1962). *Biochem. J.* **82**, 457.

Hopkins, R. P. & Young, L. (1966). *Biochem. J.* **98**, 19.

Sims, P. (1966). *Biochem. J.* **98**, 215.

Investigation on the Effects of Cigarette Smoke on Rabbit Alveolar Macrophages

By G. M. POWELL* and G. M. GREEN. (*Department of Medicine, University of Vermont, Burlington, Vt. 05401, U.S.A.*)

Alveolar macrophages occupy a functional position of primary importance in pulmonary clearance, since they are capable of the uptake, transport and elimination of particles, micro-organisms and fluids through the processes of phagocytosis, pinocytosis and digestion. Green & Carolin (1967) have shown that the presence of the filtered gas phase of cigarette smoke has a very marked inhibitory effect with respect to the phagocytic function of rabbit alveolar macrophages. Green (1968) has also shown that the cells could be protected from the effects of

* Present address: Department of Biochemistry, University College, P.O. Box 78, Cardiff CF1 1XL, U.K.